U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER
TRANMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		KATO18
		U S APPLICATION NO (If known, see 37 CFR 1 5) 09/937375
INTERNATIONAL APPLICATION NO. PCT/JP00/01533	INTERNATIONAL FILING DATE March 14, 2000	PRIORITY CLAIMED March 23, 1999
TITLE OF INVENTION GENE THERAPEUTICS		
APPLICANT(S) FOR DO/EO/US **I. KATO et al.**		

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [xx] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C 371
3. [xx] This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [xx] The US has been elected in a Demand by the expiration of 19 months from the priority date (PCT Article 31).
5. [xx] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not transmitted by the International Bureau).
   b. [xx] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [xx] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. [xx] Amendments to the claims of the International Application under PCT Article 19 (35 U.S C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [xx] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U S.C. 371(c)(3))
9. [xx] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An Assignment document for recording A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [xx] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [xx] Other items or information:
    [ ] Courtesy copy of the International Application as filed.
    [xx] Courtesy copy of the first page of the International Publication (WO 00/56368).
    [ ] Courtesy copy of the International Preliminary Examination Report. There were no annexes. [or Annexes are attached but are not to be used for initial examination in this case.]
    [ ] **Courtesy copy of the International Preliminary Examination Report with annexes containing pages ____ to be substituted for original specification pages ____ and claims _____ to be substituted for original claims ____ for examination in this case.**
    [xx] Formal drawings, 1 sheets, Figure 1.
    [xx] Courtesy Copy of the International Search Report.
    [ ] Applicant claims small entity status. See 37 CFR 1 27.
    [xx] The application is (or will be) assigned to: Takara Shuzo Co., Ltd., whose address is 609, Takenaka-cho, Fushimi-ku, Kyoto-shi, Kyoto 612-8061, Japan.

U.S. APPLICATION NO (If known, see 37 CFR 1 5)	International Application No	Attorney's Docket No
09/937375	PCT/JP00/01533	KATO18

17. [xx] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a)(1) –(5):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1 445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO..... .... ..**$1000.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.....**$860.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... ....**$710.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... .....**$690.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**

	CALCULATIONS PTO USE ONLY
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$

Claims as Originally Presented	Number Filed	Number Extra	Rate	
Total Claims	51 - 20	31	X $18.00	$ 558.00
Independent Claims	6 - 3	3	X $80.00	$ 240.00
Multiple Dependent Claims (if applicable)			+$270.00	$ 270.00
TOTAL OF ABOVE CALCULATIONS =				$ 1,928.00

Claims After Post Filing Prel. Amend	Number Filed	Number Extra	Rate	
Total Claims	- 20		X $18.00	$ 0
Independent Claims	- 3		X $78.00	$ 0
TOTAL OF ABOVE CALCULATIONS =				$ 1,928.00

Reduction of ½ for filing by small entity, if applicable. Applicant claims small entity status. See 37 CFR 1.27	$
SUBTOTAL =	$ 1,928.00
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).	$
TOTAL NATIONAL FEE =	$ 1,928.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3 28, 3 31). **$40.00** per property +	$
TOTAL FEES ENCLOSED =	$ 1,928.00
Amount to be: refunded	$
charged	$

a. [ ] A check in the amount of $_____ to cover the above fees is enclosed.

b. [XX] Credit Card Payment Form (PTO-2038), authorizing payment in the amount of $1,928.00, is attached.

c. [ ] Please charge my Deposit Account No **02-4035** in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

d. [**XX**] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **02-4035**. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

BROWDY AND NEIMARK, P.L.L.C.
624 NINTH STREET, N.W., SUITE 300
WASHINGTON, D.C. 20001
TEL: (202) 628-5197
FAX: (202) 737-3528

Date of this submission: **September 24, 2001**

SIGNATURE

Sheridan Neimark
NAME

20,520
REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Ikunoshin KATO et al.	)	Art Unit:
IA No.: PCT/JP00/01533	)	
IA Filed: March 14, 2000	)	Washington, D.C.
U.S. App. No.: (Not Yet Assigned)	)	
National Filing Date: (Not Yet Received)	)	September 24, 2001
For: GENE THERAPEUTICS	)	Docket No.: KATO=18

PRELIMINARY AMENDMENT

Honorable Commissioner for Patents and Trademarks
Washington, D.C. 20231

Sir:

Contemporaneous with the filing of this case and prior to calculation of the filing fee, kindly amend as follows:

IN THE SPECIFICATION

After the title please insert the following paragraph:

REFERENCE TO RELATED APPLICATIONS

The present application is the national stage under 35 U.S.C. §371 of international application PCT/JP00/01533, filed March 14, 2000 which designated the United States, and which application was not published in the English language.--

REMARKS

The above amendment to the specification is being made to insert reference to the PCT application of which the present case is a U.S. national stage

Favorable consideration and allowance are earnestly solicited.

Respectfully submitted,
BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant

By: ______________________
Sheridan Neimark
Registration No. 20,520

SN:wrd/edg
Telephone No.: (202) 628-5197
Facsimile No.: (202) 737-3528
F:\,A\Aoyb\Kato18\PTO\Preliminary Amendment.doc

DESCRIPTION

GENE THERAPEUTICS

Technical Field

The present invention relates to a composition and a method for missile gene therapy which are useful for treatment of diseases that require gene therapy for their treatment and for selective transfer of a gene into target cells in vivo.

Background Art

About 3000 cases of gene therapies have been conducted in the world to date. The greatest technical problem concerning the gene therapy was that the efficiency of transferring a therapeutic gene into target cells, particularly into hematopoietic stem cells, is very low. Recently, the gene transfer efficiency has been remarkably improved by the use of a recombinant protein of a fibronectin fragment, CH-296 (Takara Shuzo; RetroNectin), and thus the gene therapy is gaining practicality (Nature Medicine, 2:876-882 (1996)). The recombinant RetroNectin molecule can bind both a retrovirus having a therapeutic gene being incorporated and a target cell to allow them to become adjacent each other, thereby greatly increasing the

gene transfer efficiency. It has been said that it is the most difficult to transfer a gene into human hematopoietic stem cells. However, transfer of a therapeutic gene with an efficiency of about 90% has been achieved using RetroNectin even for the human hematopoietic stem cells. RetroNectin is a single polypeptide in which a peptide that specifically binds to a hematopoietic stem cell is connected to a peptide that specifically binds to a retrovirus vector having a therapeutic gene being incorporated. The present inventors have demonstrated that the two portions in RetroNectin exhibit the same activity as that of the original RetroNectin molecule even if they are separated each other and mixed as a cocktail, and designated this method as a cocktail gene transfer method (see WO 97/18318).

The method for transferring a gene into hematopoietic stem cells using RetroNectin is an epoch-making method in that it increases the efficiency of transferring a gene into hematopoietic stem cells. This method comprises conducting gene transfer into hematopoietic stem cells in vitro and returning the hematopoietic stem cells having the transferred gene into a living body. Thus, it may be considered that the method comprising such steps is too complicated to apply it to gene therapy in some cases.

Currently, there is a need to provide a target cell-specific gene transfer method which can deal with the diversity of target cells to be used for gene therapy.

Attempts have been made to confer directivity to target cells on non-virus vectors (e.g., a vector that uses polylysine or the like as a carrier for retaining a nucleic acid) by adding a ligand having an affinity specific for the cells. However, a gene transferred by this method cannot be stably maintained in cells. Virus vectors each expressing a fusion protein of a viral envelope with a ligand having an affinity for a target cell are known. However, the intended targeting has not been accomplished in many cases because one or both of the infective function inherent in the envelop and the binding function inherent in the ligand is damaged due to the expression as a fusion. Furthermore, it was necessary to carry out complicated construction of packaging cells for every type of target cell in order to express the fused envelope. In addition, a lot of time for preparing experiments has been required to establish a packaging cell line that can provide a high-titer virus vector suspension, to confirm that a replication competent retrovirus (RCR) does not appear, and the like.

As described above, it has been desired to solve various problems still associated with the current

techniques in order to efficiently transfer a gene of interest specifically into target cells.

Objects of Invention

The main object of the present invention is to provide a therapeutic composition useful for gene therapy comprising transferring a gene in vivo, and to provide a convenient gene therapy method comprising transferring a gene specifically into target cells in vivo using said therapeutic composition.

Summary of Invention

The present invention is outlined as follows. The first aspect of the present invention relates to a composition for gene therapy used for treating a disease susceptible to gene therapy, which contains as an active ingredient an effective amount of a functional substance that has a function of having an affinity for a virus that contains a gene useful for gene therapy and a function of having an affinity specific for a target cell for which transfer of the gene is required.

The composition of the first aspect may contain an effective amount of the virus that contains a gene useful for gene therapy. For example, the virus may be contained being mixed with the functional substance.

Alternatively, the virus may be contained such that it can be mixed with the functional substance.

For the composition of the first aspect, the function of having an affinity for a virus of the functional substance is not specifically limited and is exemplified by one derived from a functional substance selected from the group consisting of anti-virus antibodies, heparin-II-binding domain of fibronectin, fibroblast growth factor, collagen and polylysine as well as functional equivalents thereof.

For the composition of the first aspect, the function of having an affinity specific for a target cell of the functional substance is not specifically limited and is exemplified by one derived from a functional substance selected from the group consisting of proteins each having an affinity for the target cell, hormones, cytokines, anti-target cell antibodies, sugar chains, carbohydrates and cells.

The second aspect of the present invention relates to a composition for gene therapy used for treating a disease susceptible to gene therapy, which contains as active ingredients an effective amount of a functional substance having an affinity for a virus that contains a gene useful for gene therapy and an effective amount of another functional substance having an affinity specific

for a target cell for which transfer of the gene is required.

The composition of the second aspect may contain an effective amount of the virus that contains a gene useful for gene therapy. For example, the virus may be contained being mixed with the functional substance having an affinity for the virus. Alternatively, the virus may be contained such that it can be mixed with the functional substance having an affinity for the virus upon use.

For the composition of the second aspect, the functional substance having an affinity for a virus is not specifically limited and is exemplified by a functional substance selected from the group consisting of anti-virus antibodies, heparin-II-binding domain of fibronectin, fibroblast growth factor, collagen and polylysine as well as functional equivalents thereof.

For the composition of the second aspect, the functional substance having an affinity specific for a target cell is not specifically limited and is exemplified by a functional substance selected from the group consisting of proteins each having an affinity for the target cell, hormones, cytokines, anti-target cell antibodies, sugar chains, carbohydrates and cells.

The third aspect of the present invention relates to a gene therapy method for treating a disease susceptible

to gene therapy, the method comprising administering as an active ingredient an effective amount of a functional substance that has a function of having an affinity for a virus that contains a gene useful for gene therapy and a function of having an affinity specific for a target cell for which transfer of the gene is required.

In the gene therapy method of the third aspect, an effective amount of the virus that contains a gene useful for gene therapy may be administered simultaneously with the composition of the present invention or at separate time.

For the gene therapy method of the third aspect, the function of having an affinity for a virus of the functional substance is not specifically limited and is exemplified by one derived from a functional substance selected from the group consisting of anti-virus antibodies, heparin-II-binding domain of fibronectin, fibroblast growth factor, collagen and polylysine as well as functional equivalents thereof.

For the gene therapy method of the third aspect, the function of having an affinity specific for a target cell of the functional substance is not specifically limited and is exemplified by one derived from a functional substance selected from the group consisting of proteins each having an affinity for the target cell, hormones,

cytokines, anti-target cell antibodies, sugar chains, carbohydrates and cells.

The fourth aspect of the present invention relates to a gene therapy method for treating a disease susceptible to gene therapy, the method comprising administering as active ingredients an effective amount of a functional substance having an affinity for a virus that contains a gene useful for gene therapy and an effective amount of another functional substance having an affinity specific for a target cell for which transfer of the gene is required.

In the gene therapy method of the fourth aspect, an effective amount of the virus that contains a gene useful for gene therapy may be administered simultaneously with the composition of the present invention or at separate time.

For the gene therapy method of the fourth aspect, the functional substance having an affinity for a virus is not specifically limited and is exemplified by a functional substance selected from the group consisting of anti-virus antibodies, heparin-II-binding domain of fibronectin, fibroblast growth factor, collagen and polylysine as well as functional equivalents thereof.

For the gene therapy method of the fourth aspect, the functional substance having an affinity specific for a

target cell is not specifically limited and is exemplified by a functional substance selected from the group consisting of proteins each having an affinity for the target cell, hormones, cytokines, anti-target cell antibodies, sugar chains, carbohydrates and metabolites.

The fifth aspect of the present invention relates to use of an effective amount of a functional substance that has a function of having an affinity for a virus that contains a gene useful for gene therapy and a function of having an affinity specific for a target cell for which transfer of the gene is required, for the manufacture of a composition for gene therapy for treating a disease susceptible to gene therapy.

The sixth aspect of the present invention relates to use of an effective amount of a functional substance having an affinity for a virus that contains a gene useful for gene therapy and an effective amount of another functional substance having an affinity specific for a target cell for which transfer of the gene is required, for the manufacture of a composition for gene therapy for treating a disease susceptible to gene therapy.

For the composition of the first or second aspect, the gene therapy method of the third or fourth aspect, or the use of the fifth or sixth aspect, the target cell for gene transfer is not specifically limited and is

exemplified by a hematopoietic stem cell, a blood cell, a leukocyte, a lymphocyte, a T cell, a tumor-infiltrating lymphocyte, a B cell or a cancer cell.

For the composition of the first or second aspect, the gene therapy method of the third or fourth aspect, or the use of the fifth or sixth aspect, the gene to be transferred into the target cell is not specifically limited as long as it can be used for the purpose of gene therapy. A protein encoded by the transferred gene is a therapeutic protein which is expressed upon expression of the gene in the cell in an amount sufficient for the treatment. The protein is exemplified by an enzyme in a living body or a cytokine.

For the composition of the first or second aspect, the gene therapy method of the third or fourth aspect, or the use of the fifth or sixth aspect, the virus that can be used is not specifically limited as long as it can be clinically used as therapeutic means. A virus vector for which the safety has been confirmed can be used. The virus vector is exemplified by a retrovirus vector, an adenovirus vector, an adeno-associated virus vector or a vaccinia virus vector. It may be selected on the basis of its infectivity to the target cell or gene transfer efficiency.

The present inventors have found that utilization of a function having an affinity to a target cell and a

function having an affinity for a virus enables optional selection of a target cell to be used for in vivo gene transfer, efficient gene transfer into the target cell utilizing a virus, and in vivo targeting of gene transfer, or a missile gene therapy, which was previously difficult. Thus, the present invention has been completed.

Brief Description of Drawings

Figure 1 illustrates the efficiency of gene transfer using HL-60 cell as a vehicle.

Detailed Description of the Invention

The present invention is described in detail below.

The virus vector used in the missile gene therapy using the therapeutic composition of the present invention is not specifically limited. Known virus vectors usually used for gene transfer such as a retrovirus vector, an adenovirus vector, an adeno-associated virus vector or a vaccinia virus vector are used. A recombinant retrovirus vector is preferably used in the present invention. In particular, a replication-defective recombinant retrovirus vector is preferable. The ability of replication of such a vector is eliminated such that it cannot autonomously replicate in infected cells and, therefore, the vector is

non-pathogenic. The vector can invade into a host cell such as a vertebrate cell (particularly, a mammalian cell) and stably integrate a foreign gene useful for gene therapy inserted within the vector into the chromosomal DNA.

In the present invention, the gene to be transferred into target cells in vivo can be used being inserted into a recombinant retrovirus vector such that it is expressed under the control of an appropriate promoter, for example, a promoter present in the virus vector or a foreign promoter. In addition, another regulatory element (e.g., an enhancer sequence or a terminator sequence) that cooperates with a promoter and/or a transcription initiation site may be present in the vector in order to accomplish efficient transcription of the gene. Furthermore, a promoter, a transcription initiation site and another regulatory element cooperating with them that control expression in a site-specific manner (in an organ, around tumor, etc.) may be incorporated into the vector to further increase the specificity of gene expression at the target site. The gene to be transferred may be a naturally occurring gene or an artificially prepared gene. Alternatively, the gene may be one in which DNA molecules of different origins are joined together by ligation or other means known in the art.

One can select any gene of which the transfer

into target cells in vivo is desired as the gene to be inserted into the virus vector. For example, a gene encoding an enzyme or a protein associated with the disease to be treated, an intracellular antibody (see, for example, WO 94/02610), a growth factor, an antisense nucleic acid, a ribozyme, a false primer (see, for example, WO 90/13641) or the like can be used as the gene.

Examples of the functional substances having affinities for viruses used in the present invention include, but are not limited to, anti-virus antibodies, heparin-II-binding domain of fibronectin, fibroblast growth factor, type V collagen and polylysine. Also, substances functionally equivalent to these functional substances such as a functional substance having heparin-binding domain can be used. They may be derived from such functional substances. In this context, "derived from a functional substance" means that a functional site of a functional substance having an affinity for a virus is included in the molecule of the functional substance to be used. An affinity is a conception that includes an ability to bind to a virus and an ability to adhere to a cell. The affinity for a virus of the functional substance used in the present invention makes it possible to target a virus to a specific cell, or an organ or a tissue containing the cell, and to conduct gene therapy comprising transferring a

gene in vivo into a specific cell.

An antibody that has an affinity specific for a virus is particularly useful for specifically and efficiently transferring a gene into specific cells. The antibody that can be used in the present invention is not limited to a specific one. An antibody that recognizes an antigen on the surface of a virus for gene transfer can be appropriately selected for use. Such an antibody can be produced according to known methods. Alternatively, many currently commercially available antibodies can also be used. The antibody may be either a monoclonal antibody or polyclonal a antibody as long as it has desired properties such as an ability to bind to a virus to be used. Additionally, an antibody or a derivative of an antibody modified using known techniques such as a humanized antibody, a Fab fragment or a single-chain antibody can also be used.

Examples of the functional substances having affinities for target cells used in the present invention include, but are not limited to, proteins each having an affinity for the cell, hormones, cytokines, antibodies against cell surface antigens, polysaccharides, glycoproteins, glycolipids, sugar chains derived from glycoproteins or glycolipids, metabolites of the target cells and cells. They may be cell-binding sites derived

from such functional substances. In this context, "derived from a functional substance" means that a functional site of a functional substance having an affinity for a target cell is included in the molecule of the functional substance to be used. An affinity for a target cell is a conception that includes an ability to bind to a target cell and an ability to adhere to a cell. The affinity for a target cell of the functional substance used in the present invention makes it possible to target a virus to a specific cell, or an organ or a tissue containing the cell, and to conduct gene therapy comprising transferring a gene in vivo into a specific cell.

An antibody that specifically binds to a target cell is particularly useful for efficiently transferring a gene into specific target cells. The anti-target cell antibody that can be used in the present invention is not limited to a specific one. An antibody against an antigen expressed on target cells into which a gene is to be transferred can be appropriately selected for use. Such an antibody can be produced according to known methods. Alternatively, many currently commercially available antibodies can also be used. The antibody may be either a monoclonal antibody or a polyclonal antibody as long as it has desired properties such as specificity for the target cell. Additionally, an antibody or a derivative of an

antibody modified using known techniques such as a humanized antibody, a Fab fragment or a single-chain antibody can also be used.

Expression of respective leukocyte antigens (known as CD antigens) on various cells has been studied in detail. Thus, a gene can be transferred into target cells with high specificity by selecting an antibody that recognizes a CD antigen expressed on the target cells of interest and using it in the gene transfer method of the present invention. For example, gene transfer can be directed to helper T cells by using an anti-CD4 antibody, or to hematopoietic stem cells by using an anti-CD34 antibody.

Furthermore, a protein having an activity of adhering to a cell such as fibronectin, laminin, collagen or vitronectin can be used as a functional substance having an affinity for a target cell. The functional substance may be a fragment thereof as long as it has an activity of binding to a target cell.

A glycoprotein, laminin, is useful for efficiently transferring a gene into various target cells such as blood cells. The sugar chain of laminin plays an important role in gene transfer using laminin. Therefore, a sugar chain released from laminin according to a known method can also be used as a functional substance.

Furthermore, a glycoprotein having a high mannose type N-linked sugar chain like laminin, or a sugar chain released therefrom or chemically synthesized can also be used in the present invention. Additionally, a substance, such as a protein, having the above-mentioned sugar chain being attached thereto can be used. For example, a functional substance having an affinity for a retrovirus and having the sugar chain being attached thereto can be preferably used for gene transfer.

The functional substance as described above can be obtained from naturally occurring materials, prepared artificially (for example, using recombinant DNA techniques or chemical synthesis techniques), or prepared by combining a naturally occurring substance and an artificially prepared substance.

Fibronectin or a fragment thereof used in the method of the present invention can be prepared in a substantially pure form from naturally occurring materials according to methods as described, for example, in J. Biol. Chem., 256:7277 (1981); J. Cell. Biol., 102:449 (1986); or J. Cell. Biol., 105:489 (1987). The fibronectin or the fragment thereof can be prepared utilizing recombinant DNA techniques as described in United States Patent No. 5,198,423. Specifically, a fibronectin fragment containing heparin-II domain, which is a retrovirus-binding site, such

as recombinant polypeptides including CH-296 (RetroNectin), H-271, H-296 and CH-271 as well as the method for obtaining them are described in detail in the publication of the patent. These fragments can be obtained by culturing Escherichia coli strains deposited under accession numbers FERM P-10721 (H-296) (the date of the original deposit: May 12, 1989), FERM BP-2799 (CH-271) (the date of the original deposit: May 12, 1989), FERM BP-2800 (CH-296) (the date of the original deposit: May 12, 1989) and FERM BP-2264 (H-271) (the date of the original deposit: January 30, 1989) at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry, 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan as described in the publication. In addition, fragments that can be typically derived from these fragments can be prepared by modifying the plasmids harbored in these Escherichia coli strains using known recombinant DNA techniques.

Among the above-mentioned fibronectin fragments, CH-296 and CH-271 have ligands for VLA-5, and CH-296 and H-296 have ligands for VLA-4. Thus, they are useful for targeting into cells expressing VLA-5 or VLA-4. For example, a functional substance having a ligand for VLA-4 is useful for transferring a gene into hematopoietic stem cells.

A cell can be used as a functional substance having an affinity to a target cell. Certain cells have affinities specific for organs, tissues or cells. Thus, the cell is useful as a vehicle for infecting a target cell with a virus vector in vivo. Targeting of a gene into a target cell using a cell as a vehicle is exemplified by the following.

1. Gene transfer using vascular endothelial cell as vehicle

A vascular endothelial cell has a nature of being accumulated specifically at a site at which a blood vessel is newly formed. Targeting of a gene using a vascular endothelial cell as a vehicle can be conducted utilizing this nature.

Cancer cells induce vascularization around them for their growth, take up nutrients and excrete wastes through the formed blood vessels. Cancer can be treated by transporting a virus vector to a site of vascularization around the cancer cells utilizing the nature of the vascular endothelial cell as described above. For example, a suicide gene such as HSV-TK may be transferred as a therapeutic gene to directly attack a cancerous tissue. Alternatively, a gene that inhibits vascularization may be transferred to inhibit the uptake of nutrients by cancer cells and regress the cancer. Side effect observed for

such a therapy for cancer is less than that observed for conventional chemotherapy or radiotherapy. The physical burden to a patient due to surgery is dramatically reduced by using simple treatment comprising inoculation with the composition for gene therapy of the present invention.

It is preferable to promote the development of collateral circulation pathway in order to overcome the ischemic state observed after blood vessel bypass surgery for cerebral infarction or myocardial infarction. In this case, the ischemic state is ameliorated by transferring a gene involved in promotion of vascularization to a site of vascularization around the site of surgery using a vascular endothelial cell as a vehicle.

2. Gene transfer into inflamed tissue using inflammatory cell as vehicle

In case of allergic inflammation such as bronchial asthma, inflammatory cells from blood vessel lumen adhere to blood vessel wall, migrate through vascular endothelial cells, and then move into stroma to cause inflammation in respiratory tract mucous membrane. A therapy can be conducted utilizing this nature of being accumulated at an inflammation site of an inflammatory cell. Inflammatory cells that can be used as vehicles include eosinophils, mast cells and lymphocytes. For example, upon adhesion of inflammatory cells to blood vessel wall which

is the first step of accumulation, if a gene involved in inhibition of adhesion is transferred into vascular endothelial cells, the adhesion of inflammatory cells is inhibited and the accumulation does not take place thereafter.

3. Gene transfer into bone marrow microenvironment using hematopoietic stem cell as vehicle

A hematopoietic stem cell has a nature of homing into bone marrow microenvironment. Targeting of a gene can be conducted utilizing this nature. When hematopoietic stem cells home into bone marrow microenvironment along with a virus vector, a useful gene can be transferred to bone marrow microenvironment including adjacent other hematopoietic stem cells and cells composing the bone marrow microenvironment such as stromal cells.

4. Gene transfer into brain tumor using brain endothelial cell as vehicle

Targeting to a site of brain tumor can be conducted by binding a virus vector to endothelial cells derived from brain. In particular, a retrovirus vector has a nature of infecting dividing cells with high efficiency. Thus, it can transport a healing gene specifically into brain tumor without infecting non-dividing normal cells surrounding tumor.

5. Gene transfer using cell capable of

regenerating tissue as vehicle

Recently, regeneration of blood vessels using bone marrow cells was reported, and treatment of myocardial infarction utilizing this observation has been conducted. Blood flow in cardiac muscle in infarcted state is improved by injecting bone marrow cells into the cardiac muscle. If bone marrow cells are allowed to transport a virus vector having a useful gene (e.g., a vascularization-promoting gene) utilizing this nature, the ability of the bone marrow cells to regenerate blood vessel is dramatically increased due to synergistic effect with the transferred gene. Furthermore, bone marrow cells are capable of differentiating and regenerating into bone, cartilage, tendon, fat cells, skeletal muscle and stromal cells as mesenchymal stem cells are. Thus, bone marrow cells can be utilized for promotion of regeneration of tissues or cells, site-specific treatment and the like by using the bone marrow cells for transporting a therapeutic gene suitable for the purpose.

The functional substances used in the present invention include one composed of a functional substance having an affinity for a virus and a functional substance having an affinity for a target cell. For example, the functional substances are exemplified by a functional substance consisting of an anti-target cell antibody having

an affinity specific for a target cell and an anti-virus antibody having an affinity specific for a virus, a functional substance consisting of a sugar chain having an affinity specific for a target cell and an anti-virus antibody having an affinity specific for a virus, and a functional substance consisting of a cell having an affinity specific for a target cell and a polypeptide having an affinity specific for a virus. A gene can be transferred into specific cells in a living body in which various types of cells coexist using such a functional substance. In particular, sugar chains are said to be the faces of cells because they determine diverse properties of cells and cells recognize and interact each other through various sugar chains. Thus, targeting of gene transfer utilizing this specificity of sugar chain enables the most precise missile gene therapy in vivo when it is used along with an antibody which has a specific binding ability.

The composition for gene therapy of the present invention using a cell as a vehicle is exemplified by one in which a functional substance having a affinity for a virus vector is bound to a cell having an affinity specific for a target cell. Methods used for binding functional substances to cells include a method in which a functional substance is chemically bound to a cell and a method in which a functional substance that has both of an affinity

for a virus vector and an affinity specific for a cell to be used as a vehicle is used.

Furthermore, a cell that has an affinity specific for a virus vector and an affinity specific for a target cell can be utilized as a vehicle. Native cells that inherently have both of the above-mentioned properties can be used as such cells. Alternatively, the cell may have one or both of the two affinities being artificially conferred. An affinity specific for a target cell can be conferred by forcing the cell to express a functional substance having an affinity specific for the target cells (e.g., a ligand for a receptor expressed on the target cell or an antibody against a surface antigen on the target cell) on the surface of the cell. In addition, an affinity for a virus vector can be similarly conferred by expressing a functional substance having an affinity specific for the virus vector on the surface of the vehicle cell.

Vehicle cells prepared from the patient to be administered with the composition for gene therapy of the present invention are not eliminated by immunity or the like. Thus, they are particularly preferable for therapeutic purpose. If it is impossible to prepare vehicle cells from a patient, cells derived from another individual or another animal species may be used. In this case, if the cells are treated with radiation or a drug

beforehand, the cells do not grow and disappear when cells begin to divide after a certain period of time from the administration into the living body. Such cells are sufficiently functional for the purpose of transporting a virus vector.

Additionally, gene transfer efficiency can be further increased by utilizing the interaction (e.g., signal transduction) between the vehicle cell and the target cell to produce a state in which the target cell becomes susceptible to infection with the virus vector, for example, to progress cell cycle.

A composition for gene therapy used for treating a disease susceptible to gene therapy can be manufactured using as an active ingredient an effective amount of a functional substance that has a function of having an affinity for a virus that contains a gene useful for gene therapy and a function of having an affinity specific for a target cell for which transfer of the gene is required. Furthermore, a composition for gene therapy used for treating a disease susceptible to gene therapy can be manufactured using as active ingredients an effective amount of a functional substance having an affinity for a virus that contains a gene useful for gene therapy and an effective amount of another functional substance having an affinity specific for a target cell for which transfer of

the gene is required.

The therapeutic composition of the present invention can be prepared by using an effective amount of the above-mentioned functional substance as its active ingredient, and formulating it with a known pharmaceutical carrier. The composition can be administrated as an injectable preparation or a drip.

A dosage of the therapeutic composition is appropriately determined and varies depending on the particular dosage form, administration route and purpose as well as age, weight and conditions of a patient to be treated. In general, a daily dosage for an adult person is 10 μg to 200 mg/kg in terms of the amount of the active ingredient contained in the formulation. Of course, the dosage can vary depending on various factors. Therefore, in some cases, a less dosage than the above may be sufficient but, in other cases, a dosage more than the above may be required.

The present invention provides a gene therapy method comprising administering as an active ingredient an effective amount of the therapeutic composition of the present invention.

In the gene therapy method of the present invention, a functional substance having an affinity for a virus may be administered being bound to an effective

amount of a virus containing a gene useful for gene therapy. Alternatively, a functional substance having an affinity for a virus may be administered such that it binds to the virus due to its affinity in vivo. In either case, the mode of administration is determined such that a gene is efficiently transferred into target cells in vivo.

Although it is not intended to limit the present invention, it is preferable to select a method for administration suitable for the composition for gene therapy of the present invention to reach target cells.

Examples of cells to be used as a target for gene transfer according to the present invention include, but are not limited to, stem cells, hematopoietic cells, non-adhesive low-density mononuclear cells, adhesive cells, bone marrow cells, hematopoietic stem cells, peripheral blood stem cells, umbilical cord blood cells, fetal hematopoietic stem cells, embryogenic stem cells, embryonic cells, primordial germ cells, oocytes, oogonia, ova, spermatocytes, sperms, CD34+ cells, c-kit+ cells, pluripotent hematopoietic progenitor cells, unipotent hematopoietic progenitor cells, erythroid precursor cells, lymphoid mother cells, mature blood cells, blood cells, leukocytes, lymphocytes, B cells, T cells, tumor-infiltrating lymphocytes, fibroblasts, neuroblasts, neurocytes, endothelial cells, vascular endothelial cells,

hepatocytes, myoblasts, skeletal muscle cells, smooth muscle cells, cancer cells, myeloma cells and leukemia cells.

Some of gene therapies using hematopoietic stem cells as target cells are for complementing a deficient or abnormal gene in a patient. Examples thereof include the gene therapy for adenosine deaminase (ADA) deficiency (see United States Patent No. 5399346) and Gaucher's disease. In addition, a drug resistance gene may be transferred into hematopoietic stem cells in order to alleviate the damage of hematopoietic cells due to the chemotherapeutic agents used for the treatment of cancer or leukemia, for example.

Furthermore, a therapeutic method in which a suicide gene such as thymidine kinase gene is transferred into cancer cells and then a drug is administered to kill the cells has been studied as a gene therapy for cancer (Science, 256:1550-1552 (1992)). In addition, attempts are made to treat AIDS using a gene therapy. In this case, the following procedure is considered. In the procedure, a gene encoding a nucleic acid molecule (e.g., an antisense nucleic acid or a ribozyme) which interferes with the replication or the gene expression of human immunodeficiency virus (HIV) is transferred into T cells which can be infected with the causal agent of AIDS, HIV [e.g., J. Virol., 69:4045-4052 (1995)]. The target cell-

specific gene transfer according to the present invention can increase the efficiencies of gene therapies as described above.

As described above in detail, a disease that requires gene therapy for its treatment can be treated by specifically transferring a gene into target cells in vivo using the therapeutic composition and the therapeutic method of the present invention. No acute toxicity is observed when the therapeutic composition of the present invention is administered at a physiologically effective concentration into a living body

Examples

The following Examples illustrate the present invention in more detail, but are not to be construed to limit the scope thereof.

Example 1

(1) A vector PGK-hADA (Nature Medicine, 2:876-882 (1996)), a PGK vector containing human ADA gene (hADA) produced by EPHA-5-producer cells (Nature Medicine, 2:876-882 (1996)), and a injectable preparation of RetroNectin as described below in Example 2 were injected into caudal vein of a C3H/HeJ mouse (8 weeks old, purchased from Japan SLC). Transduction of hematopoietic stem cells was analyzed as described in Nature Medicine, 2:876-882 (1996) by examining

the expression of the transduced human ADA cDNA in the mouse having a transferred gene. Specifically, the presence of human ADA protein in peripheral blood cells from the mouse was confirmed by ADA isozyme analysis which uses cellulose acetate electrophoresis for detection. The examination was conducted at the beginning of the fourth month after the transplantation and repeated every month.

(2) Analysis of transduced bone marrow from the transplanted mouse using the isozyme analysis after nine months confirmed the expression of human ADA cDNA for a mouse administered with RetroNectin and the vector PGK-hADA. Human ADA was not detected for a control mouse.

Example 2

RetroNectin (CH-296, Takara Shuzo) was dissolved in injectable water at a concentration of 2 mg/ml. The solution was equilibrated with saline to prepare injectable preparations.

Example 3: Gene transfer using HL-60 cell as vehicle

A polypeptide CH-271 was prepared as follows. Briefly, Escherichia coli HB101/pCH101 (FERM BP-2799) was cultured according to the method as described in United States Patent No. 5,198,423. CH-271 was obtained from the culture.

Human leukemia HL-60 cells (purchased from

Dainippon Pharmaceutical) were suspended in D-MEM medium (Bio Whittaker) containing 10% fetal calf serum (FCS, Bio Whittaker) at a concentration 2 x 10^6 cells/ml. RetroNectin™ (Takara Shuzo) or CH-271 was added to 1 ml of the cell suspension at a final concentration of 100 µg/ml. A control group to which no such functional substance was added was provided.

100 µl of a solution containing 6.23 x 10^6 cfu/ml of an ecotropic retrovirus vector having an enhanced green fluorescent protein (EGFP) gene (pLEIN (Clontech), prepared using GP+E-86 cells (ATCC CRL-9642)) was added to the cell. The mixture was incubated at 37°C for 30 minutes in a 5% CO_2 incubator. After incubation, the cells were washed twice by centrifugation in D-MEM medium containing 10% FCS to remove the virus vector which did not adsorb to the cells. After the washing by centrifugation, the cells were suspended in 1 ml of D-MEM medium containing 10% FCS.

2 x 10^6 of the thus-obtained HL-60 cells were added to a 6-well cell culture plate (Falcon) in which 2 x 10^5 of NIH/3T3 cells (ATCC CRL-1658) had been cultured. The cells were incubated at 37°C for 2 days in a 5% CO_2 incubator. After incubation, NIH/3T3 cells adhered to the plate were collected. EGFP-expressing cells were analyzed by flow cytometry using FACSVantage (Becton Dickinson) at an excitation wavelength of 488 nm and an emission

wavelength of 515-545 nm. The gene transfer efficiency (the ratio of EGFP-expressing cells to total cells) was then calculated. The experimental results are shown in Figure 1.

As shown in Figure 1, significant expression of the EGFP gene derived from the GP+E-86/EGFP retrovirus vector was observed only for the group in which RetroNectin (CH-296) was added to HL-60 cells, indicating that gene transfer took place. Specifically, it was demonstrated that the retrovirus vector could be adsorbed to HL-60 cells via RetroNectin, approach NIH/3T3 cells as target cells along with HL-60 cells, and then infect the target cells. The virus vector adsorbed to the cells via RetroNectin was not detached after centrifugation or washing, and did not lose its infectivity upon adsorption. As described above, a vehicle cell capable of adsorbing a virus could be conveniently prepared only by adding RetroNectin to a cell suspension.

RetroNectin has a ligand (CS-1) for VLA-4, which is expressed on HL-60 cells, in addition to a ligand for VLA-5. On the other hand, CH-271 has a ligand for VLA-5 of which the expression level on HL-60 is low. It is considered that this difference reflects the difference in the gene transfer efficiency. These results show that it is possible to specifically confer an affinity for a virus

on the vehicle cells of interest even in a state in which plural types of cells coexist by appropriately selecting a functional substance having an affinity for the virus to be used in combination with the cells (e.g., a fibronectin fragment).

Example 4: Gene transfer using vascular endothelial cell as vehicle

RetroNectin was added at a final concentration of 100 µg/ml to 200 µl of D-MEM medium (Bio Whittaker, supplemented with 10% FBS) containing 5 x 10^5 of vascular endothelial cells (HUVECs, purchased from Bio Whittaker). A control group to which RetroNectin was not added was provided.

200 µl of a solution containing GP+E-86/EGFP retrovirus at a concentration of 7.75 x 10^6 cfu/ml was added to the cells. The mixture was incubated at 37°C for 30 minutes in a 5% CO_2 incubator. After incubation, the cells were washed twice by centrifugation in D-MEM medium containing 10% FCS to remove the virus vector which did not adsorb to the cells. After the washing by centrifugation, the cells were suspended in 100 µl of D-MEM medium containing 10% FCS. 5 x 10^5 of HUVEC cells prepared as described above were mixed with 1 x 10^5 L1210 cells (purchased from Dainippon Pharmaceutical) in 500 µl of RPMI 1640 medium (Bio Whittaker, supplemented with 10% FBS) and

transferred to a 24-well plate (Falcon). The cells were incubated at 37°C for 4 days in a 5% CO_2 incubator.

When the cells were examined under a fluorescence microscope after cultivation, a cluster of HUVECs surrounded by L1210 cells was observed, demonstrating that HUVECs had an affinity for L1210 cells. Furthermore, fluorescence from EGFP was observed for L1210 cells surrounding HUVECs, demonstrating that gene transfer took place in these cells. For the cells subjected to the above-mentioned procedure without the addition of RetroNectin, surrounding of HUVECs by L1210 cells was observed, but fluorescence was not observed.

From the above, it was demonstrated that a gene can be targeted into target cells using HUVECs and a functional substance such as RetroNectin in combination.

Example 5: Homing of vascular endothelial cell

Using Adenovirus Expression Vector Kit (Takara Shuzo), an adenovirus vector AxCAiLacZ which contains a control plasmid having a lacZ gene attached to the kit, pAxCAiLacZ, was prepared. HUVECs cultured in a 10-cm plate (Falcon) almost to confluence were infected with the adenovirus vector AxCAiLacZ at m.o.i. = 10, and the cultivation was continued. The cells collected 3 days after the infection were designated as LacZ-HUVECs.

1×10^6 cells of a mouse fibrosarcoma cell line

Meth-A (distributed by the Institute of Physical and Chemical Research (RIKEN), RCB 0464) were subcutaneously transplanted into a SCID mouse (obtained from Clea Japan). 2 x 10^6 of LacZ-HUVECs were inoculated through caudal vein 5 days after the transplantation. Tumor, peritoneum (together with abdominal wall) for which vascularization was observed at sites adjacent to the tumor, and various organs (liver, spleen, heart, kidney and lung) were removed from the mouse 7 days after the inoculation. Each of the above was stained using X-gal (Takara Shuzo) to examine the localization of LacZ-HUVECs. Furthermore, a portion of LacZ-HUVECs was subjected to cultivation in a plate at the same time of the inoculation into the mouse and stained with X-gal when the tumor, peritoneum and organs were stained in order to confirm that LacZ-HUVECs had the lacZ gene.

LacZ-HUVECs cultured in the plate were stained blue with X-gal, confirming that they harbored the lacZ gene. Portions of the removed tumor and peritoneum were stained blue with X-gal. In particular, a line-shaped blue staining was observed along the sites of vascularization in the peritoneum, confirming that LacZ-HUVECs were localized at the sites of vascularization. In addition, blue staining was also observed for a portion of the tumor. Thus, it was demonstrated that the administered HUVECs were

selectively accumulated at the sites of vascularization.

As described above, it was demonstrated that HUVECs can be used as a vehicle for transferring a gene to a site of tumorigenesis and a site of vascularization.

Industrial Applicability

The present invention provides a therapy which enables targeting of gene transfer into target cells in vivo, which transfers a gene specifically into target cells of interest, and, consequently, which is useful for treatment of diseases susceptible to gene therapy, as well as a composition for the therapy. Furthermore, the present invention provides a gene therapy method comprising administering said therapeutic composition and a gene therapy method comprising transferring a gene into target cells in vivo.

CLAIMS

1. A composition for gene therapy used for treating a disease susceptible to gene therapy, which contains as an active ingredient an effective amount of a functional substance that has a function of having an affinity for a virus that contains a gene useful for gene therapy and a function of having an affinity specific for a target cell for which transfer of the gene is required.

2. The composition according to claim 1, which contains an effective amount of the virus that contains a gene useful for gene therapy.

3. The composition according to claim 1 or 2, wherein the function of having an affinity for a virus is derived from a functional substance selected from the group consisting of anti-virus antibodies, heparin-II-binding domain of fibronectin, fibroblast growth factor, collagen and polylysine as well as functional equivalents thereof.

4. The composition according to any one of claims 1 to 3, wherein the function of having an affinity specific for a target cell is derived from a functional substance selected from the group consisting of proteins each having an affinity for the target cell, hormones, cytokines, anti-target cell antibodies, sugar chains, carbohydrates and cells.

5. The composition according to any one of claims 1 to 4, wherein the functional substance is a functional substance having an affinity specific for a target cell derived from a cell selected from the group consisting of vascular endothelial cells, inflammatory cells, hematopoietic stem cells, brain endothelial cells and bone marrow cells.

6. The composition according to claim 1, wherein the functional substance is a cell having an affinity specific for a target cell.

7. The composition according to claim 6, wherein the functional substance is a cell selected from the group consisting of vascular endothelial cells, inflammatory cells, hematopoietic stem cells, brain endothelial cells and bone marrow cells.

8. A composition for gene therapy used for treating a disease susceptible to gene therapy, which contains as active ingredients an effective amount of a functional substance having an affinity for a virus that contains a gene useful for gene therapy and an effective amount of another functional substance having an affinity specific for a target cell for which transfer of the gene is required.

9. The composition according to claim 8, which contains an effective amount of the virus that contains a

gene useful for gene therapy.

10. The composition according to claim 8 or 9, wherein the functional substance having an affinity for a virus is a functional substance selected from the group consisting of anti-virus antibodies, heparin-II-binding domain of fibronectin, fibroblast growth factor, collagen and polylysine as well as functional equivalents thereof.

11. The composition according to any one of claims 8 to 10, wherein the functional substance having an affinity specific for a target cell is a functional substance selected from the group consisting of proteins each having an affinity for the target cell, hormones, cytokines, anti-target cell antibodies, sugar chains, carbohydrates and cells.

12. The composition according to claim 11, wherein the functional substance having an affinity specific for a target cell is a cell having an affinity specific for a target cell.

13. The composition according to claim 12, wherein the functional substance having an affinity specific for a target cell is a cell selected from the group consisting of vascular endothelial cells, inflammatory cells, hematopoietic stem cells, brain endothelial cells and bone marrow cells.

14. A gene therapy method for treating a disease

susceptible to gene therapy, the method comprising administering as an active ingredient an effective amount of a functional substance that has a function of having an affinity for a virus that contains a gene useful for gene therapy and a function of having an affinity specific for a target cell for which transfer of the gene is required.

15. The gene therapy method according to claim 14, which comprises administering an effective amount of the virus that contains a gene useful for gene therapy.

16. The gene therapy method according to claim 14 or 15, wherein the function of having an affinity for a virus is derived from a functional substance selected from the group consisting of anti-virus antibodies, heparin-II-binding domain of fibronectin, fibroblast growth factor, collagen and polylysine as well as functional equivalents thereof.

17. The gene therapy method according to any one of claims 14 to 16, wherein the function of having an affinity specific for a target cell is derived from a functional substance selected from the group consisting of proteins each having an affinity for the target cell, hormones, cytokines, anti-target cell antibodies, sugar chains, carbohydrates and cells.

18. The gene therapy method according to any one of claims 14 to 17, wherein the functional substance is a

functional substance having an affinity specific for a target cell derived from a cell selected from the group consisting of vascular endothelial cells, inflammatory cells, hematopoietic stem cells, brain endothelial cells and bone marrow cells.

19. The gene therapy method according to claim 14, wherein the functional substance is a cell having an affinity specific for a target cell.

20. The gene therapy method according to claim 19, wherein the functional substance is a cell selected from the group consisting of vascular endothelial cells, inflammatory cells, hematopoietic stem cells, brain endothelial cells and bone marrow cells.

21. A gene therapy method for treating a disease susceptible to gene therapy, the method comprising administering as active ingredients an effective amount of a functional substance having an affinity for a virus that contains a gene useful for gene therapy and an effective amount of another functional substance having an affinity specific for a target cell for which transfer of the gene is required.

22. The gene therapy method according to claim 21, which comprises administering an effective amount of the virus that contains a gene useful for gene therapy.

23. The gene therapy method according to claim

21 or 22, wherein the functional substance having an affinity for a virus is a functional substance selected from the group consisting of anti-virus antibodies, heparin-II-binding domain of fibronectin, fibroblast growth factor, collagen and polylysine as well as functional equivalents thereof.

24. The gene therapy method according to any one of claims 21 to 23, wherein the functional substance having an affinity specific for a target cell is a functional substance selected from the group consisting of proteins each having an affinity for the target cell, hormones, cytokines, anti-target cell antibodies, sugar chains, carbohydrates and cells.

25. The gene therapy method according to claim 21, wherein the functional substance having an affinity specific for a target cell is a cell having an affinity specific for a target cell.

26. The gene therapy method according to claim 25, wherein the functional substance having an affinity specific for a target cell is a cell selected from the group consisting of vascular endothelial cells, inflammatory cells, hematopoietic stem cells, brain endothelial cells and bone marrow cells.

27. Use of an effective amount of a functional substance that has a function of having an affinity for a

virus that contains a gene useful for gene therapy and a function of having an affinity specific for a target cell for which transfer of the gene is required, for the manufacture of a composition for gene therapy for treating a disease susceptible to gene therapy.

28. The use according to claim 27, wherein the composition contains an effective amount of the virus that contains a gene useful for gene therapy.

29. The use according to claim 27 or 28, wherein the function of having an affinity for a virus is derived from a functional substance selected from the group consisting of anti-virus antibodies, heparin-II-binding domain of fibronectin, fibroblast growth factor, collagen and polylysine as well as functional equivalents thereof.

30. The use according to any one of claims 27 to 29, wherein the function of having an affinity specific for a target cell is derived from a functional substance selected from the group consisting of proteins each having an affinity for the target cell, hormones, cytokines, anti-target cell antibodies, sugar chains, carbohydrates and cells.

31. The use according to any one of claims 27 to 30, wherein the functional substance is a functional substance having an affinity specific for a target cell derived from a cell selected from the group consisting of

vascular endothelial cells, inflammatory cells, hematopoietic stem cells, brain endothelial cells and bone marrow cells.

32. The use according to claim 27, wherein the functional substance is a cell having an affinity specific for a target cell.

33. The use according to claim 32, wherein the functional substance is a cell selected from the group consisting of vascular endothelial cells, inflammatory cells, hematopoietic stem cells, brain endothelial cells and bone marrow cells.

34. Use of an effective amount of a functional substance having an affinity for a virus that contains a gene useful for gene therapy and an effective amount of another functional substance having an affinity specific for a target cell for which transfer of the gene is required, for the manufacture of a composition for gene therapy for treating a disease susceptible to gene therapy.

35. The use according to claim 34, wherein the composition contains an effective amount of the virus that contains a gene useful for gene therapy.

36. The use according to claim 34 or 35, wherein the functional substance having an affinity for a virus is a functional substance selected from the group consisting of anti-virus antibodies, heparin-II-binding domain of

fibronectin, fibroblast growth factor, collagen and polylysine as well as functional equivalents thereof.

37. The use according to any one of claims 34 to 36, wherein the functional substance having an affinity specific for a target cell is a functional substance selected from the group consisting of proteins each having an affinity for the target cell, hormones, cytokines, anti-target cell antibodies, sugar chains, carbohydrates and cells.

38. The use according to claim 34, wherein the functional substance having an affinity specific for a target cell is a cell having an affinity specific for a target cell.

39. The use according to claim 38, wherein the functional substance having an affinity specific for a target cell is a cell selected from the group consisting of vascular endothelial cells, inflammatory cells, hematopoietic stem cells, brain endothelial cells and bone marrow cells.

40. The composition, the gene therapy method or the use according to any one of claims 1 to 39, wherein the target cell is a cell selected from the group consisting of hematopoietic stem cells, blood cells, leukocytes, lymphocytes, T cells, tumor-infiltrating lymphocytes, B cells and cancer cells.

41. The composition, the gene therapy method or the use according to any one of claims 1 to 40, wherein the target cell is a cell selected from the group consisting of hematopoietic stem cells, blood cells, leukocytes, lymphocytes, T cells, tumor-infiltrating lymphocytes, B cells and cancer cells.

42. The composition, the gene therapy method or the use according to any one of claims 1 to 41, wherein a protein encoded by the transferred gene is a therapeutic protein which is expressed upon expression of the gene in the target cell in an amount sufficient for the treatment.

43. The composition, the gene therapy method or the use according to 42, wherein the protein is an enzyme or a cytokine.

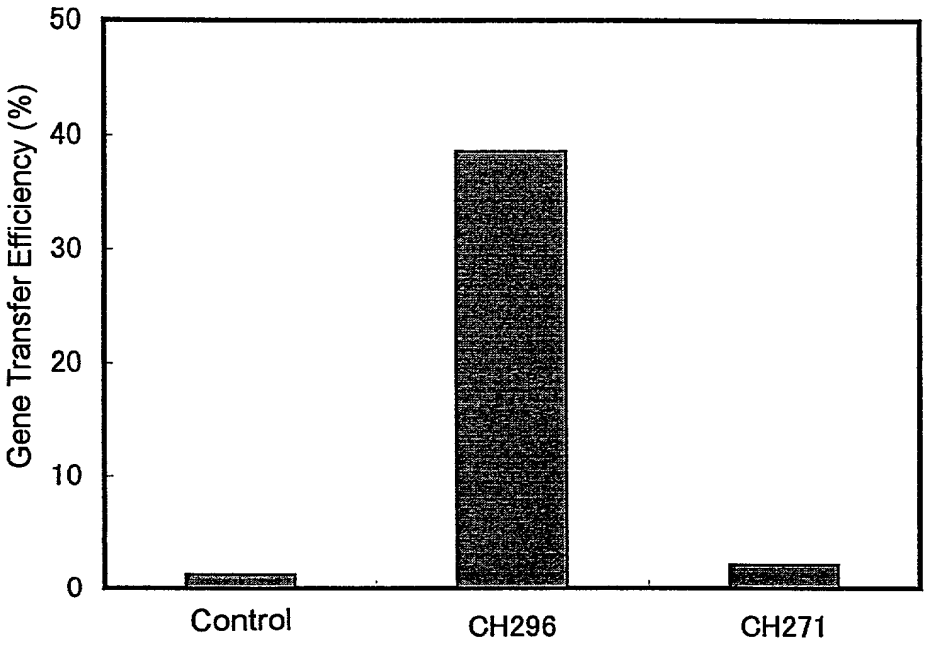
44. The composition, the gene therapy method or the use according to any one of claims 1 to 43, wherein the virus is a virus vector.

45. The composition, the gene therapy method or the use according to any one of claims 1 to 44, wherein the virus is a retrovirus vector, an adenovirus vector, an adeno-associated virus vector or a vaccinia virus vector.

ABSTRACT

Gene therapeutics to be used in treating diseases showing sensitivity to gene therapy, characterized by containing as the active ingredient an efficacious amount of a functional substance which has a function of having an affinity for a virus containing a gene usable in the gene therapy and another function of having an affinity specific for a target cell with a need for the gene transfer, or an efficacious amount of a functional substance which has an affinity for the above virus and an efficacious amount of another functional substance which has an affinity specific for the above cell.

Fig. 1



Combined Declaration for Patent Application and Power of Attorney

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

GENE THERAPEUTICS

the specification of which (check one)

[ ] is attached hereto;
[ ] was filed in the United States under 35 U.S.C. §111 on _____, as U.S. Appln. No. _____*; or
[X] was/will be filed in the U.S. under 35 U.S.C. §371 by entry into the U.S. national stage of an international (PCT) application, PCT/JP00/01533; filed 14/03/2000, entry requested on _____*; national stage application received U.S. Appln. No. _____*; §371/§102(e) date _____* (* if known)

and was amended on _____ (if applicable).
(include dates of amendments under PCT Art. 19 and 34 if PCT)

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above; and I acknowledge the duty to disclose to the Patent and Trademark Office (PTO) all information known by me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §§ 119 and 365 of any prior foreign application(s) for patent or inventor's certificate, or prior PCT application(s) designating a country other than the U.S., listed below with the "Yes" box checked and have also identified below any such application having a filing date before that of the application on which priority is claimed:

(Number)	(Country)	(Day Month Year Filed)	[X] YES	[ ] NO
078591/1999	Japan	23/03/1999		
(Number)	(Country)	(Day Month Year Filed)	[ ] YES	[ ] NO

I hereby claim the benefit under 35 U.S.C. §120 of any prior U.S. non-provisional application(s) or prior PCT application(s) designating the U.S. listed below, or under §119(e) of any prior U.S. provisional applications listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in such U.S. or PCT application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the PTO all information as defined in 37 C.F.R. §1.56(a) which occurred between the filing date of the prior application and the national filing date of this application:

(Application No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)
(Application No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)
(Application No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)

As a named inventor, I hereby appoint the following registered practioners to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

All of the practioners associated with Customer Number 001444

Direct all correspondence to the address associated with Customer Number 001444; i.e.,

BROWDY AND NEIMARK, P.L.L.C.
624 Ninth Street, N.W.
Washington, D.C. 20001-5303
(202) 628-5197

The undersigned hereby authorizes the U.S. Attorneys or Agents appointed herein to accept and follow instructions from AOYAMA & PARTNERS as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. Attorneys or Agents and the undersigned. In the event of a change of the persons from whom instructions may be taken, the U.S. Attorneys or Agents appointed herein will be so notified by the undersigned.

Page 2 of 2 Pages
Title: GENE THERAPEUTICS
Atty. Docket:
U.S. Application filed _____, Serial No. _____
PCT Application filed March 14, 2000, Serial No. PCT/JP00/01533

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00

FULL NAME OF FIRST INVENTOR	INVENTOR'S SIGNATURE	DATE
Ikunoshin KATO	Ikunoshin Kato	14/08/2001
RESIDENT: Uji-shi, Kyoto Japan JPX		CITIZENSHIP: Japan
POST OFFICE ADDRESS: 1-1-150, Nanryo-cho, Uji-shi, Kyoto 611-0028 Japan		

2-00

FULL NAME OF SECOND JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
Kiyozo ASADA	Kiyozo Asada	14/08/2001
RESIDENT: Koka-gun, Shiga Japan JPX		CITIZENSHIP: Japan
POST OFFICE ADDRESS: 3-20-9, Kibogaoka, Konan-cho, Koka-gun, Shiga 520-3333 Japan		

3-00

FULL NAME OF THIRD JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
Mitsuhiro UENO	Mitsuhiro Ueno	14/08/2001
RESIDENT: Kusatsu-shi, Shiga Japan JPX		CITIZENSHIP: Japan
POST OFFICE ADDRESS: 6-28, Nishishibukawa 2-chome, Kusatsu-shi, Shiga 525-0025 Japan		

4-00

FULL NAME OF FOURTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
Kimikazu HASHINO	Kimikazu Hashino	14/08/2001
RESIDENT: Takatsuki-shi, Osaka Japan JPX		CITIZENSHIP: Japan
POST OFFICE ADDRESS: 27-3, Akeno-cho, Takatsuki-shi, Osaka 569-0082 Japan		

5-00

FULL NAME OF FIFTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
Hirofumi YOSHIOKA	Hirofumi Yoshioka	14/08/2001
RESIDENT: Kusatsu-shi, Shiga Japan JPX		CITIZENSHIP: Japan
POST OFFICE ADDRESS: 311, Hamoparesu-Kusatsu, 12-1, Nishishibukawa 2-chome, Kusatsu-shi, Shiga 525-0025 Japan		

6-00

FULL NAME OF SIXTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
Keiji TANAKA	Keiji Tanaka	14/08/2001
RESIDENT: Otsu-shi, Shiga Japan JPX		CITIZENSHIP: Japan
POST OFFICE ADDRESS: 4-10, Kojogaoka, Otsu-shi, Shiga 520-0821 Japan		

FULL NAME OF SEVENTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENT		CITIZENSHIP
POST OFFICE ADDRESS		

ALL INVENTORS MUST REVIEW APPLICATION AND DECLARATION BEFORE SIGNING. ALL ALTERATIONS MUST BE INITIALED AND DATED BY ALL INVENTORS PRIOR TO EXECUTION. NO ALTERATIONS CAN BE MADE AFTER THE DECLARATION IS SIGNED. ALL PAGES OF DECLARATION MUST BE SEEN BY ALL INVENTORS.